Efficiency of EMB Medium Combined with MALDI-TOF in Isolating and Identifying Coliform Lactose Fermenters and Non-Lactose Fermenters from Wastewater

Abstract :

In Côte d'Ivoire, the sewer network is largely underdeveloped, resulting in the absence or incompleteness of sanitation infrastructure in many urban and rural areas. Untreated wastewater, often discharged into rivers and other sources of drinking water, spreads waterborne diseases, particularly affecting children and vulnerable populations. Coliforms, key indicators of the microbiological quality of water, play a crucial role in assessing health risks. This study focuses on the diversity of lactose-fermenting and non-lactose-fermenting coliforms in the wastewater of Abidjan, using MALDI-TOF mass spectrometry for rapid and accurate identification. Out of 80 samples, we found a preponderance of lactose-fermenting coliforms, notably Klebsiella pneumoniae and Escherichia coli, highlighting the need for better wastewater management strategies to protect public health and limit antimicrobial resistance.

Keywords: Coliforms lactose fermenters, non-lactose fermenters, MALDI-TOF, wasted water, sewage

1. Introduction

Water contamination by pathogenic microorganisms is a major public health concern, particularly in densely populated urban areas like Abidjan, Côte d'Ivoire (3). In Côte d'Ivoire, the sewage network is largely underdeveloped (9). In many urban and rural areas, sanitation infrastructure is absent or incomplete (9). Major cities like Abidjan, although better equipped, still lack a sufficient wastewater management system to meet the growing demand due to rapid urbanization (2). The absence of wastewater treatment has direct repercussions on public health (2).Untreated wastewater is often discharged into rivers, lakes, and oceans, thus contaminating drinking water sources (13). This contamination is responsible for numerous waterborne diseases such as cholera, typhoid, and dysentery, which particularly affect children and vulnerable populations (13). Among these microorganisms, coliforms, including lactose-fermenting and non-lactose-fermenting coliforms, play a crucial role as indicators of the microbiological quality of water (10). Accurate detection and identification of these coliforms in wastewater are essential to assess health risks and implement appropriate management measures (10). Coliforms are a diverse group of bacteria widely used as indicators of fecal contamination (10, 18). Lactose-fermenting coliforms can ferment lactose, producing acid and gas, while non-lactose-fermenting coliforms lack this capability (10). The distinction between these two groups is important as it helps better understand the origin and pathogenic potential of contaminations (10). Generally, the presence of lactose-fermenting coliforms often indicates recent contamination by human or animal fecal matter, posing a direct risk to public health (10). Evaluating the diversity of coliforms in wastewater can provide valuable information on the efficiency of wastewater treatment systems and the potential presence of antibiotic-resistant bacteria (15). The use of MALDI-TOF mass spectrometry (Matrix-Assisted Laser Desorption/Ionization-Time of Flight) is an innovative and effective method for the rapid and accurate identification of bacteria (21). This technology generates protein profiles specific to each bacterial species, thereby facilitating the precise and rapid identification of coliforms present in wastewater samples (21). In this context, this study aims to evaluate the diversity of lactose-fermenting and non-lactose-fermenting coliforms in the wastewater of Abidjan using MALDI-TOF mass spectrometry. The results obtained will not only provide a better understanding of the microbiological composition of wastewater but also offer valuable insights for improving wastewater management and treatment strategies in this region.

2. Material and Methods

Wastewater was sampled from Cocodypalmeraiearea, where open sewers are located behind residences. This area, being a convergence point for domestic wastewater and household waste, represents a key site for measuring the presence and concentration of various pathogenic microorganisms. A microbiological analysis was performed to identify the bacteria responsible for waterborne diseases. Urban wastewater samples were collected in sterile containers from various collection points, mainly large collectors and sewers near residences. This study was conducted over a period of four weeksduring the rainy season. The protocol used for the detection of coliforms in wastewater follows Ricker CR, Eldred BJ (7).
Twenty wastewater samples were taken using a sterile dipper and placed in 1-liter bottles. The samples were transported to the laboratory in coolers with ice packs, under controlled conditions to prevent contamination. The isolation of strains from urban wastewater involves several steps, including liquid enrichment and culture on selective media. For enrichment, 1 ml of wastewater is added to 9 ml of EPT broth. The inoculated broth is incubated at 37°C for 24 hours. For isolation, after 24 hours of incubation in EPT broth, aliquots of 10 µl are spread on EMB (Eosin Methylene Blue) agar plates. The plates are incubated at 37°C for 24 hours. This medium is used to differentiate between lactose-fermenting bacteria (lactose-fermenting) and non-lactose-fermenting bacteria (non-lactose-fermenting). Lactose-fermenting bacteria appear colored on EMB, while non-lactose-fermenting bacteria remain colorless or translucent. From these 20 samples, 80 isolates were obtained. Bacteria showing colors were more numerous than those that were colorless. Thus, we selected 55 colored strains and 25 colorless strains. Among the colored isolates, we selected 25 black colonies with metallic sheen, 25 large pink mucoid colonies, and 5 other smooth pink colonies. We then took 25 other transparent colonies. These colonies were subcultured on ordinary agar for identification with MALDI-TOF.
MALDI-TOF mass spectrometry was used to identify the bacterial isolates. Each sample was prepared by applying a chemical matrix and then subjected to a laser for ionization, followed by detection of the generated ions, creating a unique spectrum for each species. The obtained spectra were compared to a reference database for species identification.

3. Results

In this study, we analyzed 80 samples to determine the distribution of lactose-fermenting and non-lactose-fermenting coliforms. The identified species include *Klebsiella pneumoniae*, *Escherichia coli, Aeromonas punctata, Acinetobacter radioresistens, Enterobacter kobei, Enterobacter cloacae, Proteus mirabilis,* and *Morganella morganii.* The classification of lactose-fermenting and non-lactose-fermenting coliforms is crucial for understanding their role in ecosystems and their potential impact on human health. We isolated 25 strains of *Klebsiella pneumoniae*, 25 strains of *Escherichia coli*, 2 strains of *Aeromonas punctata*, 1 strain of *Acinetobacter radioresistens*, 3 strains of *Enterobacter kobei*, 2 strains of *Enterobacter cloacae*, 10 strains of *Proteus mirabilis*, and 12 strains of *Morganella morganii*. The lactose-fermenting species include *Klebsiella pneumoniae*, *Escherichia coli, Enterobacter kobei,* and *Enterobacter cloacae*, while the non-lactose-fermenting species include *Aeromonas punctata*, *Acinetobacter radioresistens, Proteus mirabilis,* and*Morganella morganii*. The distribution of lactose-fermenting and non-lactose-fermenting coliforms shows a predominance of lactose-fermenters in the samples analyzed. *Klebsiella pneumoniae* and *Escherichia coli* alone represent half of the total isolates, confirming their dominant presence in the studied environments.

3.1. Statistic analyse

To properly analyze these results, we need to use tables and statistical tests. In this case we used t - student test.

Student's t-test for Comparing Groups

The Student's t-test is employed to compare the means of two independent groups to determine if there is a statistically significant difference between these means. This test can be performed using Software Version X.

Hypotheses of the Student's t-test

- **Null hypothesis (H0)**: There is no significant difference between the means of the two groups.
- **Alternative hypothesis (H1)**: There is a significant difference between the means of the two groups.

For a hypothetical example, we obtained an approximate t-value of $t \approx 1.025$.

The degrees of freedom (df) for the t-test are approximated by $df \approx 5.79$. To determine statistical significance, we compare our t-value with the critical values from the Student's t-distribution for our degrees of freedom and significance level ($\alpha = .05$). For $df \approx 6$ and $\alpha = .05$, the critical value is approximately 2.447.

Since our t-value (1.025) is less than 2.447, we do not have enough evidence to reject the null hypothesis. Therefore, there is no significant difference between the means of the two groups.

Table 1. Distribution of bacterial species according to the number of samples and the percentage

SPECIES	**NUMBER OF STRAINS**	**PERCENTAGE**
KLEBSIELLA PNEUMONAE	25	31,5
ESCHERICHIA COLI	25	31,5
ENTEROBACTER KOBEI	3	3,75
ENTEROBACTER CLOACAE	2	2,5
MORGANELLA MORGANII	12	15
PROTEUS MIRABILIS	10	12,5
ACINETOBACTER RADIORESISTENS	1	1,25
AEROMONAS PUNCTATA	2	2,5
TOTAL	40	100

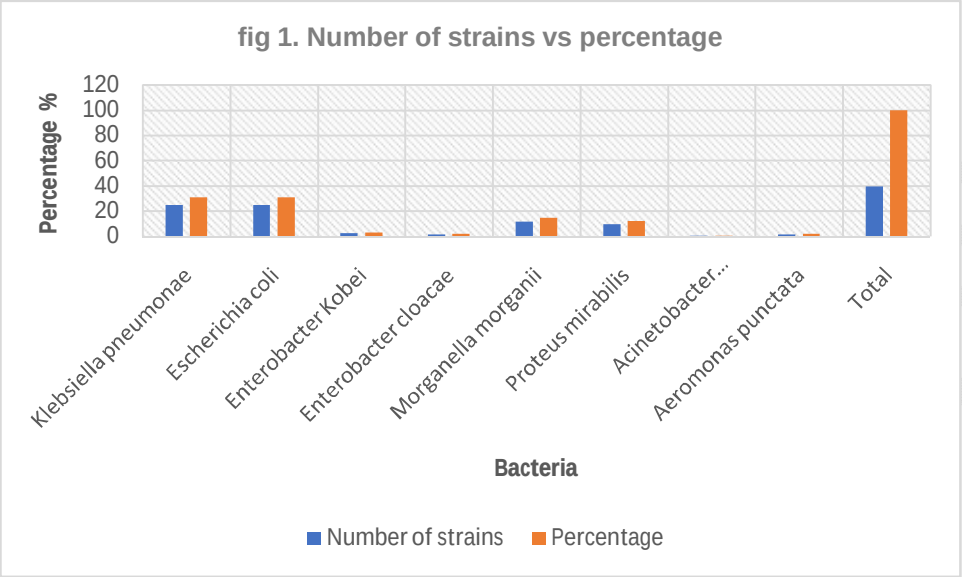
Table 2. Lactose fermentersstrains

SPECIES	NUMBER OF STRAINS	(%)
KLEBSIELLA PNEUMONAE	25	31,5
ESCHERICHIA COLI	25	31,5
ENTEROBACTER KOBEI	3	3,75
ENTEROBACTER CLOACAE	2	2,5
TOTAL	**55**	**69,25**

Table 3. Non lactose fermentersstrains

SPECIES	**NUMBER OF STRAINS**	**(%)**
MORGANELLA MORGANII	12	15
PROTEUS MIRABILIS	10	12,5

ACINETOBACTER RADIORESISTENS	1	1,25
AEROMONAS PUNCTATA	2	2,5
TOTAL	**25**	**31,25**



4. Discussion

The analysis of the 80 samples revealed a notable diversity of coliforms, encompassing both lactose-fermenting and non-lactose-fermenting species. Among the identified species, *Klebsiella pneumoniae* and *Escherichia coli* were the most prevalent (14, 8). This prevalence is consistent with previous studies highlighting the dominance of these species in various environments, particularly wastewater and contaminated soils (17).

Klebsiella pneumoniae is particularly concerning due to its ability to cause nosocomial infections and develop antibiotic resistance (14). Resistance mechanisms, including the production of extended-spectrum beta-lactamases (ESBL) and carbapenemases, make this bacterium particularly difficult to treat (14). Additionally, *Klebsiella pneumoniae* is associated with a variety of infections, including pulmonary, urinary, and septic infections, making it a significant public health threat (14).

Escherichia coli (E. coli), though often harmless as an intestinal commensal, includes pathogenic strains that can cause urinary tract infections, gastrointestinal infections, and septicemias (8). The genetic diversity of E. coli allows this bacterium to adapt to different environments and hosts, thereby increasing its pathogenic potential (8). Toxin-producing E. coli strains, such as enterohemorrhagic strains, are particularly concerning due to their association with severe outbreaks (20).

The identified non-lactose-fermenting species, such as *Aeromonas punctata* and *Acinetobacter radioresistens*, also have significant clinical implications (5, 16). *Aeromonas punctata* is known for its opportunistic infections, particularly in immunocompromised individuals (5). Infections caused by *Aeromonas* can include gastroenteritis, septicemia, and wound infections (5). Moreover, *Aeromonas* species often possess intrinsic antibiotic resistance mechanisms, complicating their treatment (5).

Acinetobacter radioresistens, though less studied, is part of a genus recognized for its multiple resistances and persistence in hospital environments (16). Acinetobacter spp. are often responsible for nosocomial infections, including ventilator-associated pneumonia and wound infections (16). The ability of Acinetobacter to survive on inanimate surfaces for long periods contributes to its spread in hospitals (6).

The identification of *Enterobacter kobei*and *Enterobacter cloacae* reinforces the idea of environmental contamination, as these bacteria are frequently found in soil and freshwater samples (12). *Enterobacter cloacae* is particularly concerning due to its association with hospital infections and its ability to produce extended-spectrum beta-lactamases, limiting available therapeutic options (12).

Proteus mirabilis and *Morganella morganii*, though less frequently encountered, are nonetheless significant due to their involvement in urinary tract infections and septicemias (11, 15). Proteus mirabilis is a common cause of complicated urinary tract infections, often associated with urinary stone formation due to its ability to hydrolyze urea (15). *Morganella morganii*, though rare, can cause severe infections, including septicemias, particularly in immunocompromised patients (11).

These results underscore the need for continuous monitoring and rigorous management of potential sources of microbiological contamination, particularly in sensitive environments such as healthcare facilities and drinking water systems. The presence of these coliforms, combined with their pathogenic potential, represents a significant health risk that must be addressed through risk management strategies and preventive interventions (3).

Conclusion

This studyhighlighted the diversity of lactofermenting and non-lactofermentingcoliformspresent in the samplesanalyzed. The speciesidentified, including Klebsiella pneumoniae, Escherichia coli, and other non-lactofermentingcoliforms, illustrate the complexity of microbialecosystems and the challenges they pose in terms of public health. The prevalence of thesebacteria in environmentalsamples highlights the need for rigorous control measures to preventassociated infections and limit the spread of antimicrobialresistance. The diversity of speciesdetected and their distribution alsosuggest avenues for future research, including the study of resistance and transmission mechanisms, as well as the impact of environmental practices on the prevalence of thesemicroorganisms. In conclusion, thisstudycontributes to the understanding of coliformdynamics in the environment and theirpotentialinvolvement in human infections, highlighting the importance of an integratedapproach to environmentalhealth management.

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